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**BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES**

Application Number: 09/710,058
Filing Date: November 10, 2000
Appellant(s): ANDERSON ET AL.



James S. Keddie
For Appellant

**EXAMINER'S ANSWER**

This is in response to the appeal brief filed 3/8/07 appealing from the Office action mailed 10/13/06.

## Table of Contents

**(1) Real Party in Interest**

A statement identifying by name the real party in interest is contained in the brief.

**(2) Related Appeals and Interferences**

The examiner is not aware of any related appeals, interferences, or judicial proceedings which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

**(3) Status of Claims**

The statement of the status of claims contained in the brief is correct.

**(4) Status of Amendments After Final**

The appellant's statement of the status of amendments after final rejection contained in the brief is correct.

**(5) Summary of Claimed Subject Matter**

The summary of claimed subject matter contained in the brief is correct.

**(6) Grounds of Rejection to be Reviewed on Appeal**

The appellant's statement of the grounds of rejection to be reviewed on appeal is correct.

**(7) Claims Appendix**

The copy of the appealed claims contained in the Appendix to the brief is correct.

**(8) Evidence Relied Upon**

Art Rejection References:

**Bryan et al.** US 6,232,107 (5/01: filed 10/98 or earlier);

**Aran et al.** Cancer Gene Therapy, Vol. 5, No. 4: 195-206. (1998);

**Zolutukhin et al.** US 5,874,304 (2/99: filed 1/96);

**Bierhuizen et al.** Biochemical and Biophysical Research Communications. Vol. 234: 371-375. 1997;

**Anderson et al.** PNAS. Vol. 93: 8508-8511. 1996;

**Result 4** DATABASE Alignment search (see "Result 4"; 1/16/03).

**(9) Grounds of Rejection**

The following ground(s) of rejection are applicable to the appealed claims:

*Bryan and Aran*

Claims 1-3 and 20 are rejected under 35 U.S.C. 103(a) as being unpatentable over **Bryan** et al. US Pat. No. 6,232,107 (5/01: filed 10/98 or earlier) with attached Result 4 DATABASE Alignment search and **Aran** et al. Cancer Gene Therapy, Vol. 5, No. 4 pages 195-206 (1998).

**Bryan et al**, disclose and claim the use (e.g. diagnostics and high throughput screening e.g. libraries) of nucleic acid molecules encoding green fluorescent proteins (e.g. bioluminscent) from the genus Renilla, including nucleotide reference Seq. ID No. 15 which is 98.4% (with best local similarity of 99.4%) homologous to elected Seq. ID No.1 (the nucleic acid sequence); differing by only one nucleotide (C vs. G). Bryan further teaches protein Seq. Id. No. 16 which

corresponds (e.g. has **100% sequence identity**) to "wild type" Renilla GFP of **Seq. Id. No.2**, as recited in the instant Claim 1. [see Reference Seq. Id 15 and attached Result 4 DATABASE Alignment search and Reference sequence Id. 16].

Bryan et al further teach host cells (e.g. present claims 1 and 3) including prokaryotic/eukaryotic (e.g. mammalian) which incorporate genetic constructs comprising a polynucleotide (e.g. cDNA) encoding a green fluorescent protein (GFP) having the amino acid sequence of SEQ ID No.2 (e.g. (sea pansy) wild-type *Renilla Mullerie* GFP) as well as the use of "human codon-optimized nucleic acid encoding a Renilla GFP" as in the present claim 20 (e.g. "The genes can be modified by substitution of codons optimized for expression in selected host cells or hosts, such as humans and other mammals ..."; See col. 5).

It is important to note that the Bryan reference, although teaching both (jellyfish) wild-type *Aequorea Victoria* GFP and (sea pansy) wild-type *Renilla Mullerie* GFP; the use of *Renilla* is strongly preferred due to the analytical problems present in the former. Particularly, *Aequorea* GFP possess two separate excitation peaks, whereas *Renilla* GFP has one excitation peak making it not ideal for use in analytical and diagonostic purposes:

"Consequently, (jellyfish) GFP mutants have been selected with the hope of **identifying mutants** that have *single excitation spectral peaks* shifted to the red (emphasis provided).

In fact **a stated purpose in constructing such mutants has been to attempt to make *A. Victoria* GFP more like the GFP from *Renilla*,** which has thus far not been cloned, but which has properties that make it far more ideal for use as an analytical tool. For many practical applications, the spectrum of Renilla GFP would be preferable to that of the Aequorea GFP, because *wavelength discrimination* between different fluorophores and detection of resonance energy transfer are easier if the component spectra are tall and narrow rather than low and broad". (emphasis provided; col.4, lines 54+).

See also '107, col. 3-5; col. 47-48;

The Bryan reference differs, if at all, from the presently claimed invention (e.g. see claims 1, 3 and 20) in failing to *explicitly teach* the use of a retrovirus as a vector.

However, in this regard, the Bryan reference teaches that a wide variety of multipurpose vectors suitable for the expression of heterologous proteins are known to those of skill in the art and are commercially available; with selection and use of such vehicles as being well within the skill of the artisan. In this regard, the Bryan vectors for use in mammalian hosts include "**recombinant *virus***", as well as plasmid and phages e.g. the use of "**retroviral** long-terminal repeats and inducible promoters from other eukaryotic expression systems".. See e.g. col. 23 (especially bottom) to col. 24; col. 59-60 (emphasis provided). Accordingly, the Bryan reference taken alone provides motivation to select the use of retroviral vectors, especially for use in mammalian host cells.

Accordingly, it would have been obvious to one of ordinary skill in the art at the time of appellant's invention to select a retroviral vector for use in a cellular host (e.g. procarytoic or mammalian) with use of a genetic construct comprising a polynucleotide (e.g. cDNA) encoding a wild-type Renilla green fluorescent protein (GFP) or a fusion thereof with a reasonable expectation of success in light of the reference's ability to express Renilla GFP and in view of the benefits of using Renilla GFP (e.g. as compared to *A. Victoria* GFP.

To the extent that further motivation to select a retroviral vector is needed and to the extent that Bryan fails to teach the incorporation of an IRES site (e.g. in present claim 2) in its fusion constructs, the Aran reference is cited.

The **Aran et al.** reference teaches the favorable use of retroviral vectors, both in vitro and in vivo including an internal ribosome entry site (IRES) for fusion constructs preferably comprising optimized, humanized (e.g. see page 204, left column for benefits of humanizing) GFP (e.g. *Aequorea victoria*); since "[T]his vector allows rapid and specific identification of the

expressed protein (e.g. MDR1 gene transfer) in living cells (e.g. mammalian cells) " (E.g. see Abstract and page 195, especially right column).

Accordingly, it would have been obvious to one of ordinary skill in the art at the time of applicants invention to utilize a retroviral vector as the Bryan "recombinant virus" vector with the use of an IRES for expressing humanized or non-humanized wild-type renilla GFP in the Bryan et al. reference in order to appreciate the benefits thereof; e.g. rapid and specific identification of the expressed protein.

*Aran, Bryan and Zolutukhin*

Claim 20 is rejected under 35 U.S.C. 103(a) as being unpatentable over the obviousness rejections using **Aran** et al and **Bryan** et al. as applied to claims 1-3 and 20 above, and, if necessary, further in view of Zolutukhin et al. US Pat. No. 5,874,304 (2/99: filed 1/96).

The above combined teaching of the Aran and Bryan references as described in the above obviousness rejection is hereby incorporated by reference in their entirety.

The combined reference teaching differs, if at all, from the presently claimed invention (e.g. claim 20) by failing to *explicitly* teach a human codon-optimized nucleic acid encoding a Renilla GFP (e.g. humanized GFP) in a retroviral vector.

However, **Zolutukhin et al** teach that utilizing human codon-optimized nucleic acid GFP in nucleic acid constructs (including fusion proteins; e.g. col. 4 corresponding to present claim 2 "first gene" terminology) serves to overcome prior art obstacles and is advantageous (e.g. improved expression in mammalian and human cells). These constructs are included in vectors

(e.g. see col. 5, examples; particularly retroviral: see patent claims, especially claims 50 and 69) contained in cells (e.g. see patent claims 71-80) in which the constructs contain:

1. GFP (particularly Renilla: see col. 1, last paragraph; col. 14 and Table 1; and especially col. 16, lines 3-15: □... spectrum of Renilla ... preferable to that of Aequorea);

2. IRES elements (e.g. see '304 patent, col. 13, line 50; claims 50 and 62).

(See also col. 1-2).

Accordingly, one of ordinary skill in the art at the time of applicant's invention would have been motivated to utilize human codon-optimized nucleic acids expressing Renilla GFP in the genetic constructs (e.g. cells/vectors comprising renilla GFP/IRES elements) rendered obvious by the combined Aran et al and Bryan et al teaching in light of the advantages thereof imparted by such humanized sequences as taught by the Zolutukhin et al. reference.

Thus, it would have been prima facie obvious to one of ordinary skill at the time of appellant's invention to modify the cellular/vector genetic constructs taught by the Aran and Bryant reference to include human codon-optimized (e.g. humanized) nucleotides encoding renilla GFP in order to obtain the advantages thereof as taught by the Zolutukhin et al. reference.

*Zolutukhin and Bryan*

Claims 1-3 and 20 are rejected under 35 U.S.C. 103(a) as being unpatentable over **Zolutukhin** et al. US Pat. No. 5,874,304 (2/99: filed 1/96) and **Bryan** et al. US Pat. No. 6,232,107 (5/01: filed 10/98 or earlier) with attached Result 4 DATABASE Alignment search.

**Zolutukhin et al** teach that utilizing human codon-optimized nucleic acid GFP in nucleic acid constructs (including fusion proteins; e.g. col. 4 corresponding to present claim 2 "first

gene" terminology) serves to overcome prior art obstacles and is advantageous (e.g. improved expression in mammalian and human cells). These constructs are included in vectors (e.g. see col. 5, examples; particularly retroviral: see patent claims, especially claims 50 and 69) contained in cells (e.g. see patent claims 71-80) in which the constructs contain:

1. GFP (particularly Renilla: see col. 1, last paragraph; col. 14 and Table 1; and especially col. 16, lines 3-15: □... spectrum of Renilla ... preferable to that of Aequorea);

2. IRES elements (e.g. see '304 patent, col. 13, line 50; claims 50 and 62).

(See also col. 1-2).

It is noteworthy that Zolutukhin teaches that (sea pansy) Renilla GFP is more preferable as a reporter than Aequorea GFP since Aequorea has two absorbance peaks whereas Renilla GFP has a single absorbance peak at 498 accordingly:

For many practical applications, the spectrum of Renilla GFP would be preferable to that of Aequorea because wavelength discrimination between different fluorophores and detection of resonance energy transfer are easier when the component spectra are tall and narrow rather than low and broad." Accordingly, Zolutukhin (like the Bryan reference) teaches mutation of Aequorea toward obtaining a single peak (e.g. like Renilla) is desired. (Zolutukhin, col. 16, especially lines 10+).

Although the Zolutukhin et al. reference teaches nucleic acid which employ the preferential use of Renilla GFP, the Zolutukhin reference differs from the presently claimed invention by failing to explicitly teach the use of a *Renilla* GFP gene sequence which encodes wild type Renilla GFP corresponding to SEQ ID NO.2.

Bryan et al disclose and claim the use (e.g. diagnostics and high throughput screening e.g. libraries) of nucleic acid molecules encoding green fluorescent proteins (e.g. bioluminscent) from the genus Renilla, including nucleotide reference Seq. ID No. 15 which is 98.4% (with best local similarity of 99.4%) homologous to elected seq. ID 1; differing by only one nucleotide (C vs. G). Bryan further teaches protein SEQ ID No. 16 which corresponds (e.g. has **100% sequence identity**) to "wild type" Renilla GFP of **SEQ ID NO.2**, as presently claimed [see the attached Result 4 DATABASE Alignment for comparison between the instant SEQ ID No.1 and the reference's SEQ ID NO. 15].

Bryan et al further teach host cells (e.g. present claims 1 and 3) including prokaryotic/eukaryotic (e.g. mammalian) which incorporate genetic constructs comprising a polynucleotide (e.g. cDNA) encoding a green fluorescent protein (GFP) having the amino acid sequence of SEQ ID 2 (e.g. (sea pansy) wild-type *Renilla Mullerie* GFP) as well as the use of "human codon-optimized nucleic acid encoding a Renilla GFP" as in present claim 20 (e.g. "The genes can be modified by substitution of codons optimized for expression in selected host cells or hosts, such as humans and other mammals ..." See col. 5). The reference further teaches the use of a fusion partner (e.g. a targeting agent as a first gene) in its genetic fusion constructs as in present claims 2 and 3. See e.g. col. 8; col. 11-15; col. 24.

Bryan et al teach the use of the bioluminescent green fluorescent proteins in cellular assays (e.g. live cells, including mammalian) and in high throughput screening systems (e.g. employing libraries) (e.g. see col. 2-3; 14).

It is important to note that the Bryan et al. reference, although teaching both (jellyfish) wild-type *Aequorea Victoria* GFP and (sea pansy) wild-type *Renilla Mullerie* GFP; the use of

*Renilla* is strongly preferred due to the analytical problems present in the former. Particularly, *Aequorea* GFP possess two separate excitation peaks, whereas *Renilla* GFP has one excitation peak making it not ideal for use in analytical and diagonostic purposes:

"Consequently, (jellyfish) GFP mutants have been selected with the hope of **identifying mutants** that have *single excitation spectral peaks* shifted to the red (emphasis provided).

In fact **a stated purpose in constructing such mutants has been to attempt to make *A. Victoria* GFP more like the GFP from *Renilla*,** which has thus far not been cloned, but which has properties that make it far more ideal for use as an analytical tool. For many practical applications, the spectrum of Renilla GFP would be preferable to that of the Aequorea GFP, because *wavelength discrimination* between different fluorophores and detection of resonance energy transfer are easier if the component spectra are tall and narrow rather than low and broad". (emphasis provided; col.4, lines 54+).

See also '107, col. 3-5; col. 47-48;

Thus, it would have been obvious to one of ordinary skill in the art at the time of applicant's invention to utilize the Bryan polynucleotide Renilla green fluorescent protein (including seq. Id 15) in the Zolutukhin reference genetic constructs since:

a. BOTH Zolutukin and Bryan teach the preferential use of Renilla GFP thus motivating the selection of the Bryan Renilla GFP obvious to one of ordinary skill in the art; and/or

b. one of ordinary skill in the art would have been motivated to select the Bryan reference Renilla sequences for purposes of performing screening assays (e.g. high throughput library screens) in order to obtain the benefits of the renilla protein in such assays as taught by the Bryan reference.

*Bierhuizen and Bryan*

Claims 1, 3, and 20-22 are rejected under 35 U.S.C. 103(a) as being obvious over **Bierhuizen** et al (Biochemical and Biophysical Research Communications. Vol. 234: 371-375;

1997), in view of **Bryan** et al (US Patent 6,232,107; 2001; Filed 3/26/1999; priority date: 3/27/1998).

**Bierhuizen** et al teach using retroviral vector to transfer Green Fluorescent Protein (GFP) into mammalian cells (see Abstract of the reference). The reference teaches both wildtype and mutant (in term of amino acid sequence) of *Aequorea Victoria* GFP were expressed in mammalian cells (see Figure 1; page 373, left col., 1st paragraph; and page 374, left col., 1st paragraph). The reference also teaches that retroviral vectors comprising various GFP constructs were generated (See page 373, left col., 1st paragraph), which would read on a retroviral vector comprising a polynucleotide encoding a green fluorescent protein. In addition, the reference teaches that FACS was used to analyze GFP expression (See Figure 1 and caption). The reference further teaches that humanized (meaning replacing Aequorea Victoria codons with human codons in the coding DNA sequence) GFP can achieve higher expression in mammalian cells (Page 371, right col., last lines of 2nd paragraph). Furthermore, the reference teaches that the purpose of the study was to evaluate the potential applicability of GFP expression as a marker for the rapid selection of retrovirally transduced mammalian cells (See page 374, left col., 1st line). The study of the reference conclusively teaches that the data showed that all variants (including the wildtype GFP) allow for flow cytometric detection (FACS) of stable GFP expression in mammalian cells and that he expression can be transferred by the MFG retroviral vector (See page 374, left col., 1st paragraph, last lines).

Bierhuizen et al do not expressly teach the Retinilla GFP with the specific amino acid sequence recited in SEQ ID No 2.

However, **Bryan** et al teach the use of Renilla GFP as described in the rejection under "Bryan and Aran", and is incorporated by reference to its entirety as set forth below. The reference teaches use (e.g. diagnostics and high throughput screening e.g. libraries) of nucleic acid molecules encoding green fluorescent proteins (e.g. bioluminscent) from the genus Renilla, including Seq. Id. No. 16 which corresponds (e.g. has 100% sequence identity) to "wild type" Renilla GFP of Seq. Id. 2, as presently claimed. Bryan et al further teach host cells (e.g. present claims 1 and 3) including prokaryotic/eukaryotic (e.g. mammalian) which incorporate genetic constructs comprising a polynucleotide (e.g. cDNA) encoding a green fluorescent protein (GFP) having the amino acid sequence of SEQ ID 2 (e.g. (sea pansy) wild-type Renilla Mullerie GFP) as well as the use of "human codon-optimized nucleic acid encoding a Renilla GFP" as in present claim 20 (e.g. "The genes can be modified by substitution of codons optimized for expression in selected host cells or hosts, such as humans and other mammals ..." . See col. 5). The reference further teaches the use of a fusion partner (e.g. a targeting agent as a first gene) in its genetic fusion constructs as in present claims 2 and 3. See e.g. col. 8; col. 11-15; col. 24. Additionally, Bryan et al teach the use of the bioluminescent green fluorescent proteins in cellular assays (e.g. live cells, including mammalian) and in high throughput screening systems (e.g. employing libraries) (e.g. see col. 2-3; 14).

It is important to note that the Bryan reference, although teaching both (jellyfish) wild-type *Aequorea Victoria* GFP and (sea pansy) wild-type *Renilla Mullerie* GFP; the use of *Renilla* is strongly preferred due to the analytical problems present in the former. Particularly, *Aequorea* GFP possess two separate excitation peaks, whereas *Renilla* GFP has one excitation peak making it not ideal for use in analytical and diagonostic purposes:

"Consequently, (jellyfish) GFP mutants have been selected with the hope of **identifying mutants** that have *single excitation spectral peaks* shifted to the red (emphasis provided).

In fact **a stated purpose in constructing such mutants has been to attempt to make *A. Victoria* GFP more like the GFP from *Renilla*,** which has thus far not been cloned, but which has properties that make it far more ideal for use as an analytical tool. For many practical applications, the spectrum of Renilla GFP would be preferable to that of the Aequorea GFP, because *wavelength discrimination* between different fluorophores and detection of resonance energy transfer are easier if the component spectra are tall and narrow rather than low and broad". (emphasis provided; col.4, lines 54+).

See also '107, col. 3-5; col. 47-48;

The Bryan reference differs, if at all, from the presently claimed invention (e.g. see claims 1, 3 and 20) in failing to *explicitly teach* the use of a retrovirus as a vector.

However, in this regard, the Bryan reference teaches that a wide variety of multipurpose vectors suitable for the expression of heterologous proteins are known to those of skill in the art and are commercially available; with selection and use of such vehicles as being well within the skill of the artisan. In this regard, the Bryan vectors for use in mammalian hosts include "**recombinant *virus***", as well as plasmid and phages e.g. the use of "**retroviral** long-terminal repeats and inducible promoters from other eukaryotic expression systems". See e.g. col. 23 (especially bottom) to col. 24; col. 59-60 (emphasis provided). Accordingly, the Bryan reference taken alone provides motivation to select the use of retroviral vectors, especially for use in mammalian host cells.

Accordingly, it would have been obvious to one of ordinary skill in the art at the time of applicant's invention to select a retroviral vector for use in a cellular host (e.g. prokaryotic or mammalian) with use of a genetic construct comprising a polynucleotide (e.g. cDNA) encoding a wild-type Renilla green fluorescent protein (GFP) or a fusion thereof with a reasonable expectation of success in light of the reference's ability to express Renilla GFP and in view of the benefits of using Renilla GFP (e.g. as compared to *A. Victoria* GFP).

Therefore, it would have been prima facie obvious for an ordinary skilled artisan to screen for generate a retroviral vector comprising a polynucleotide encoding a GFP with a specific amino acid sequence (e.g. wildtype Renilla GFP amino acid sequence). Due to the advantages taught by both Bierhuizen et al and Bryan et al that GFP allows rapid selection of retrovirally transduced mammalian cells, a person of ordinary skill in the art would have been motivated at the time of the invention to construct a retroviral vector comprising a specific GFP (e.g. Renilla GFP) for using in a mammalian gene expression system. Since the construction of retroviral vector comprising various GFPs (including wildtype, mutant, or humanized) is known in the art (such as taught by Bierhuizen et al), and the specific sequence of a Renilla GFP is known and expressable in mammalian cells (as taught by Bryan et al), an ordinary skilled artisan would have been motivated to generate a retroviral vector comprising GFP having a specific amino acid sequence and a mammalian cell comprising the retroviral vector. An ordinary skilled artisan would have reasonable expectation of success of achieving such modifications since Bierhuizen et al have demonstrated the success of generating retroviral vector comprising GFP used in mammalian cell expression.

In conclusion, the invention of the instant claims would have been prima facie obvious over Bierhuizen et al, in view of Bryan et al to one of ordinary skill in the art without evidence to the contrary.

*Bierhuizen, Bryan and Aran*

Claims 1-3, and 20-22 are rejected under 35 U.S.C. 103(a) as being obvious over **Bierhuizen** et al (Biochemical and Biophysical Research Communications. Vol. 234: 371-375;

1997), in view of **Bryan** et al (US Patent 6,232,107; 2001; Filed 3/26/1999; priority date: 3/27/1998) and further in view of **Aran** et al (Cancer Gene Therapy. Vol. 5: 195-206; 1998).

**Bierhuizen** et al teach a GFP retroviral vector and mammalian gene expression system as described supra under "Bierhuizen and Bryan", and is hereby incorporated by reference in its entirety.

**Bryan** et al teach a Renilla GFP and its uses in various gene expression system as described supra under "Bryan and Aran", and is hereby incorporated by reference in its entirety.

Both of the references do not expressly teach the expression vector comprises IRES.

However, **Aran** et al teach a retroviral vector comprising an IRES element (See Page 197, left col., 2nd paragraph). The reference also teaches that the retroviral vector comprises a GFP and is used to transducer a mammalian cell (page 197, left col., 2nd and last paragraphs). The reference further teaches the advantage of including IRES element such as the element allows efficient cap-independent translation of the downstream gene.

Therefore, it would have been prima facie obvious for an ordinary skilled artisan to screen for generate a retroviral vector comprising a polynucleotide encoding a GFP with a specific amino acid sequence (e.g. wildtype Renilla GFP amino acid sequence) and an IRES element. Due to the advantages taught by Aran et al that the inclusion of an IRES element in a retroviral vector expression system would facilitate gene expression, an ordinary skilled artisan would be motivated at the time of the invention to generate a retroviral GFP expression vector comprising an IRES element. In addition, the inclusion of an IRES element in a eukaryotic gene expression system is well known in art such as taught by Aran et al. An ordinary skilled artisan would have reasonable expectation of success of achieving such modifications since Aran et al

have demonstrated the success of generating retroviral vector comprising GFP and IRES used in mammalian cell expression system.

In conclusion, the invention of the instant claims would have been prima facie obvious over Bierhuizen et al, in view of Bryan et al and further in view of Aran et al to one of ordinary skill in the art without evidence to the contrary.

*Anderson and Bryan*

Claims 1, 3, and 20-22 are rejected under 35 U.S.C. 103(a) as being obvious over **Anderson** et al (PNAS. Vol. 93: 8508-8511; 1996), in view of **Bryan** et al (US Patent 6,232,107; 2001; Filed 3/26/1999; priority date: 3/27/1998).

**Anderson** et al teach using retroviral vector expressing a GFP in mammalian cells. The reference teaches that retroviral gene transfer was used to stably incorporate the wildtype GFP in a mammalian cell (see page 8509, left col., 1st para. under RESULTS) and expressed in mammalian cells (NIH 3T3). The reference also teaches that FACS analysis of the retroviral vector comprising the wildtype GFP transduced cells revealed a single peak on a fluorescence histogram, and there was a two fold difference in fluorescence value between infected and uninfected cells (page 8509, left col., 1st para. under RESULTS). These would read on the fluorescence of the GFP can be detected by FACS since the fluorescence of the retroviral transduced cells in the reference was detected.

Anderson et al do not expressly teach that the cDNA for the GFP is humanized, and the specific GFP amino acid sequence.

However, **Bryan** et al teaches a Renilla GFP (with specific amino acid sequence) and its uses in various gene expression systems as described supra under "Bryan and Aran", and is hereby incorporated by reference in its entirety.

Therefore, it would have been prima facie obvious for an ordinary skilled artisan to screen for generate a retroviral vector comprising a polynucleotide encoding a GFP with a specific amino acid sequence (e.g. wildtype Renilla GFP amino acid sequence) that is encoded by a humanized cDNA. Due to the advantages taught by Bryan et al that GFP allows rapid selection of retrovirally transduced mammalian cells and the motivation to use GFP in mammalian expression system as discussed supra (i.e. superior spectra property of Renilla GFP), a person of ordinary skill in the art would have been motivated at the time of the invention to construct a retroviral vector comprising a specific GFP (e.g. a humanized Renilla GFP) for using in a mammalian gene expression system. Since the construction of retroviral vector comprising various GFPs (including wildtype, mutant, or humanized) is known in the art (such as taught by Anderson et al), and the specific sequence of a Renilla GFP is known and is shown to be expressed in mammalian cells (as taught by Bryan et al), an ordinary skilled artisan would have been motivated to generate a retroviral vector comprising GFP having a specific amino acid sequence and a mammalian cell comprising the retroviral vector. An ordinary skilled artisan would have reasonable expectation of success of achieving such modifications since Anderson et al have demonstrated the success of generating retroviral vector comprising GFP used in mammalian cell expression.

**(10) Response to Argument**

*Claim Interpretation*

The instant **Claim 1** recites: "a retroviral vector comprising a polynucleotide encoding a green fluorescent protein (GFP) having the amino acid sequence of SEQ ID No:2." To simplify, the claim is drawn to a retroviral vector comprising a polynucleotide. The claim language also limits that the said polynucleotide encodes for a specific GFP, whose amino acid sequence comprises SEQ ID No:2. Thus, the scope of the instant claims encompasses any polynucleotide (DNA sequences) that encodes for the amino acid sequence of SEQ ID No:2. As discussed in the rejections listed above (see Rejection over "Bryan and Aran"), the specific amino acid sequence recited in the instant SEQ ID NO:2 is an exact match to the wild-type amino acid sequence for a *Renilla* (Sea Pansy) GFP.

The instant **Claim 2** is dependent on the instant claim 1, and recites additional nucleic acid elements (such as IRES site) comprised by the claimed "retroviral vector".

The instant **Claim 20** is ultimately dependent on claim 1, and recites the polynucleotides of Claim 1 to comprise human codon nucleic acid sequences.

The instant **Claim 3** recites a "mammalian cell" comprising the "retroviral vector" recited in the instant claim 1.

The instant **Claims 21** and **22** both recite "a mammalian cell" comprising the "retroviral vector" substantially the same to the vector recited in claim 1. The instant claim 21 has an additional limitation reciting that the "fluorescence of said GFP can be detected by FACS", which can be construed as intended use of the claimed mammalian cell. Similarly, the instant claim 22 recites "said mammalian cell is in the presence of a test agent, and wherein an effect of

said test agent is detected by detecting fluorescence of said GFP", which recitation is also construed as intended use of the claimed product.

*Pertinent Terms*

The following terms are pertinent to all discussions:

Retroviral Vector: A retroviral vector is used in the art, for example, as an artificial nucleic acid construct derived from a retrovirus, used to insert sequences into an organism's chromosomes.

Thus, the instant claimed product (a "retroviral vector") is essentially a polynucleotide vector that comprises various elements (nucleic acid sequences), as discussed in the previous Office action (mailed 10/13/06, p.9, para 2). That is the claimed invention is a retroviral vector (a polynucleotide) comprising a polynucleotide with nucleic acid sequence encoding the wildtype Renilla GFP protein.

Wildtype GFP nucleic acids: The term is generally referring to the wildtype (or naturally occurring) nucleic acid sequence for the Green Fluorescent Protein. For example, in the case of Renilla GFP, the wildtype Renilla GFP gene composed of wildtype Renilla codons is considered as the "wildtype GFP nucleic acids" for Renilla.

Wildtype GFP amino acids: The term is generally referring to the wildtype (or naturally occurring) amino acid sequence for the Green Fluorescent Protein. For example, in the case of Renilla GFP, the wildtype Renilla GFP protein is composed of wildtype Renilla amino acid

sequences regardless of the coding nucleic acid sequences. That is multiple nucleic acids (with different sequences) can encode for the same "wildtype GFP".

Human codon-optimization: Human codon optimization (or used interchangeably, Humanization of codons) is a phrase referring to the process of changing the codons (i.e. nucleic acid sequences) encoding for a protein (i.e. amino acid sequences) to nucleic acid sequences that corresponds to nucleic acid sequences that most often occur in human, but without changing the encoded amino acid sequences. For example, in the instant application, the wildtype codons (nucleic acid sequences) encoding for the wildtype GFP that naturally occur in Renilla (Sea Pansy) is replaced with codons (encoding for the same wildtype amino acid sequence) that most often used in human. That is the wildtype amino acid sequence of Renilla GFP is still the same after human codon-optimization, but the codon optimized encoding nucleic acid sequence is different from the wildtype Renilla GFP nucleic acid sequence. This interpretation of the term is consistent with the usage throughout the instant specification (e.g. Spec. pp. 69-70), as well as it is used in the art (e.g. Zolutukhin et al, discussed above; Levy et al, discussed below).

*Appellant's Main Arguments*

Appellants rebut all of the above listed art rejections (under 35 USC 103(a)) with the following arguments:

1.) Retroviral vectors encoding wild-type GFPs are shown to be inoperative at the time of filing. (Brief, p. 5, para 5);

2.) No reasonable expectation of success for modifying the cited references. (Brief, p.5, para 5).

Thus, a prima facie case obviousness cannot exist.

Appellants cited several references (Aran, Hanzano, Levy, Cheng and Anderson) to support the above assertion of "inoperability". The teaching of each listed references are discussed in detail in subsequent sections of the instant Examiner's Answer.

First, it is important to note that appellants agree with the following facts as reflected by the record:

1.) The amino acid sequence of wild-type Renilla (Sea Pansy) GFP (the instant SEQ ID NO.2) was known prior to filing of the instant application. The polynucleotides encoding for the said wild-type Renilla GFP are also known in the art, as taught by Bryan et al (US 6,232,107; SEQ ID Nos 15-16). (See Applicant's Reply, entered 7/24/06, p. 6, last para)

2.) Retroviral vectors containing altered Aequoria (Jelly fish) GFP were well known and had been successfully used prior to filling of the instant application. (See Applicant's Reply, entered 7/24/06, p. 6, last para)

3.) The superior spectral properties of wild-type Renilla GFP (over Aequoria GFP) were well known prior to filing the instant application. (See Applicant's Reply, entered 7/24/06, p. 6, last para)

Appellants reiterated these facts in the instant Appeal Brief (filed 3/8/07; p. 7, para 3):

"The Appellants agree with the Examiner in that the amino acid sequence of wild-type Renilla GFP [SEQ ID No.2] was known prior to filing of their patent application. The Appellants also agree that retroviral vectors containing altered *Aequoria* GFP were well known and had

been successfully used prior to filing their patent application. Finally, the Appellants agree that the superior spectral properties of wild-type Renilla GFP were well known prior to filing their patent application." (emphasis provided by Appellants)

Initially, it is noted that "Aequoria" (Jelly fish) GFP is not the instantly claimed "Renilla" (Sea Pansy) GFP.

Even before detailed analysis of the prior art's teachings and the "inoperable" references, the above discussion would logically lead to the conclusion that it would have been prima facie obvious for a person of ordinary skill in the art to make a retroviral vector comprising a polynucleotide that encodes for the wild-type Renilla GFP, i.e. encodes for the amino acid sequence of the instant SEQ ID No:2. It is undisputed by the appellant that retroviral vectors comprising GFP (altered), wild-type *Renilla* GFP amino acid sequence (SEQ ID No:2), and polynucleotides encoding for wild-type *Renilla* GFP are all known in the prior art. Appellants have pointed out that "the superior spectral properties of wild-type *Renilla* GFP were well known prior to filing" of the instant application as discussed above, which provides ample motivation for one of ordinary skill in the art to construct a retroviral vector comprising a polynucleotide that encodes for the wild-type Renilla GFP with superior spectral properties. Contrary to appellant's assertion, the prior art does not teach away from wild-type Renilla GFP (wild-type amino acid sequence) as demonstrated by appellant's admission of the prior art's teaching discussed above.

*Discussion of Appellant's "Supporting References"*

Applicants have cited the Aran, Hanazono, Levy, Cheng and Anderson (collectively referred to as the "Supporting References" by appellants) to show that a retroviral vector

comprising a wild-type GFP would not work (i.e. no detectable fluorescence in a retroviral-mammalian gene expression system) to indicate "inoperability" and "no reasonable expectation of success".

Overall, all the references cited by the appellants to argue for "inoperability" and/or "no reasonable expectation of success" are drawn to *Aequoria* GFP but not *Renilla* GFP as discussed in the previous Office actions mailed 3/22/06. Appellants argue that Aequoria wildtype GFP cannot be expressed in a retroviral-mammalian gene expression system. However, appellants' assertion of "inoperability" and "no expectation of success" is for the *Renilla* wildtype GFP. Contrary to appellant's assertion, the state of the art at the time of the invention was made does not teach that Renilla wildtype GFP is incompatible with the retroviral mammalian gene expression system. Therefore, the cited references do not provide support for the "inoperability" and "no expectation of success" asserted for Renilla wildtype GFP since the GFPs are from two different species.

Contrary to appellants' assertion, this viewpoint is not in conflict with the 103 art rejections set forth by the previous Office action. The art rejections are based on analysis of obviousness, where the Bryan et al reference teaches that the wild-type Renilla GFP is strongly preferred over the Aequorea GFP due to the analytical problems presented in the latter (see more detail in the above rejection over "Bryan and Aran"). This provides strong motivation for one of ordinary skill in the art at the time of the invention was made to use Renilla GFP instead of Aequorea GFP in a retroviral-mammalian gene expression system. This preference of Renilla GFP over the Aequorea GFP also provides support for "reasonable expectation of success" because the Renilla GFP would solve the analytical problem presented in Aequorea GFP.

In addition, obviousness does not require absolute predictability of success; rather, all that is required for obviousness under § 103 is a "reasonable expectation of success." In re O'Farrell, 853 F.2d at 903-904 [7 USPQ2d at 1681]. Here, a person of ordinary skill in the art would have "reasonably" expected to be successful because the Renilla GFP is shown to be spectrally superior to Aequorea GFP, and would overcome the analytical problems presented in Aequorea GFP (see Bryan, col.4, lines 54+ and col. 3-5; col. 47-48; also see the rejection over "Bryan and Aran").

Detailed analysis of each of the "supporting references":

***Anderson*** The reference teaches generating retroviral vector expressing the wildtype Aequorea GFP (Anderson. p. 8508, left col., beginning of para 2). Specifically, the Anderson reference states "Fluorescence can be detected by flow cytometry in mammalian cells... transfected with a wild-type GFP" (Anderson. p. 8508, left col., beginning of para 2). In Figure 1 of the Anderson reference (p. 8509; right col.), fluorescent signal was shown to be detected for retroviral vector comprising wildtype Aequorea GFP. Thus, the reference clearly shows that a retroviral vector comprising a polynucleotide encoding a wildtype GFP can be generated, and the wildtype (Aequorea) GFP can be successfully expressed.

Appellants cited the following statements from the Anderson reference to indicate "inoperability":

1. Anderson states "in the background section that suboptimal excitation spectra of wild type GFP "*precludes the detection of wtGFP* when a single copy of the gene is stably integrated". (Emphasis provided by appellants; Brief, p. 8, footnote 13).
2. "in the first paragraph of the result section, with reference to a population of cells infected with a retroviral vector encoding wild type Aequoria GFP, states "the difference in

*fluorescene was not sufficient* to resolve infected from uninfected cells" *[sic]* (Emphasis provided by appellants; Brief, p. 8, footnote 13).

Appellant's citation 1 is only referring to one embodiment of the reference's teaching where only "a single copy of the gene is stably integrated". The statement does not indicate that a retroviral vector comprising the gene encoding for the wildtype GFP, in general, would not express at all.

In regard to Appellant's citation 2, the complete teaching of the reference is as follows:

"While the median fluorescence value of the MGF-wtGFP-infected population was 2-fold greater than that of uninfected cells (Fig. 1A), the difference in fluorescence was not sufficient to resolve infected from uninfected cells." (p. 8509, left col., para 4, bottom; emphasis added).

(It is noted that MGF-wtGFP represents the retroviral vector used to infect the mammalian cell for GFP expression).

Although, the difference in fluorescence value between the infected and the uninfected cells are not statistically significant, the wildtype GFP still expressed as indicated by the 2-fold increase in fluorescence value.

***Levy*** The Levy reference teaches constructing retroviral vector comprising different forms (wildtype and mutant) of *Aequoria* GFP and transduction into mammalian cells. The "wildtype" GFP taught by the reference refers to "wildtype" both in terms of amino acid sequence and nucleic acid sequence. For example, the reference teaches that the "wildtype GFP gene without red-shift mutation or codon modifications" (emphasis added) exhibited little (when transiently transfected) or no (stable transfection) fluorescence (See Table 1 and Caption). The

reference further teaches that with "humanization" (human codon usage) and a single amino acid mutation, the GFP was successfully expressed and intense fluorescence was observed (See Table 1 and Caption). The conclusion quoted by appellants stating that "wildtype GFP could never be visualized" (p. 613, 1st paragraph of the Levy reference; Brief, p. 8, Footnote 11) is referring to a wildtype *Aequoria* GFP both in its coding sequence and amino acid sequence. The reference also teaches that several studies have discovered that by humanizing the wildtype codons (Levy, p. 610, right col., para 2), wild-type GFP (in term of protein sequence) can be successfully expressed in mammalian cells. The conclusion of this reference does not particularly indicate that wildtype (in term of amino acid sequence) is incompatible with retroviral-mammalian expression system. The reference, however, does provide motivation to utilize humanized GFP coding nucleic acid sequence for construction of a retroviral vector to be used in a mammalian gene expression system.

***Hanazono*** The Hanazona reference teaches construction of a retroviral vector for the mammalian expressing of **Mutant** *Aequoria* **GFPs**. The reference only teaches a mutant form of GFP, which is different from a wildtype GFP. Furthermore, although the reference does teach that no stable cell lines expressing GFP were produced, the reference teaches that a retroviral vector comprising a GFP (mutant form) was successfully produced, and the mutant GFP containing retroviral vector was successfully introduced into mammalian cells (p. 1316, para 1). The reference teaches "fluorescence of cells transfected with the GFP-containing plasmids was observed..." (p.1316, para 1, line 2), which shows that the mutant GFP contained in viral vector

was successfully introduced into the mammalian cells and mutant GFP was indeed expressed regardless how long the transfected cell lived.

***Cheng*** The Cheng reference teaches expressing various forms of *Aequoria* GFP in mammalian cells using different expression vectors (transient and retroviral vectors). Similar to the Hanazono reference, the Cheng reference teaches retroviral vector comprising mutant form of GFP (in term of amino acid sequence) that was transduced into mammalian cells (See Cheng, Figure 2 and p. 607, left col., para 2). The reference teaches that the wildtype GFP gene (nucleic acid sequence) was used to construct a transient mammalian expression vector (**not** a retroviral vector) (See Cheng, p. 606, right col., last paragraph). The reference further teaches that the wildtype GFP gene expressed in the transfected mammalian cells (See Cheng, Figure 1) as analyzed by FACS. Contrary to applicants' interpretation, the reference does not teach that the wildtype GFP is incompatible with mammalian gene expression system, but the wildtype and mutant GFP "are stable and properly processed to form functional fluorophores." (Cheng, p. 608, right col., lines 1-5 under Discussion). The reference further teaches that the "Expression of GFPs, either transiently or stably, are not detrimental to host cells." (Cheng, p. 608, right col., lines 1-5 under Discussion). Thus, the reference indicates "reasonable expectation of success" of expressing wildtype GFP, in general, in mammalian cells.

Therefore, all the "supporting references" cited by the appellants do not provide support for appellants' arguments of "inoperability" and "lack of reasonable expectation of success". These references, however, do provide ample evidence to show that various forms of GFP

(including both wildtype and mutant forms) can be successfully inserted into a retroviral vector and/or expressed in mammalian cells. These references also provide motivations to humanize (alter wildtype GFP gene to have human codons) wildtype GFP gene for expression in mammalian cells. These references have demonstrated that expressing GFPs in mammalian cells (using various expression vectors such as retroviral vector) is highly feasible and successful, which would provide motivations for one skilled in the art to generate mammalian gene expression system with different GFPs (derived from different sources or mutant forms), especially of wild-type Renilla GFP because its' "superior spectral properties" as acknowledged by the appellants.

*Discussion of Grounds of Rejections*

In combination with the general discussion of the rejection under 35 U.S.C. § 103, appellants also briefly traversed each one of the 103 rejections individually. Appellants' arguments are addressed as the followings:

**Rejection over Anderson and Bryan:**

Appellants cited the following statements from the Anderson reference to indicate "inoperability":

1. Anderson states "in the background section that suboptimal excitation spectra of wild type GFP "*precludes the detection of wtGFP* when a single copy of the gene is stably integrated". (Emphasis provided by appellants; Brief, p. 8, footnote 13).

2. "in the first paragraph of the result section, with reference to a population of cells infected with a retroviral vector encoding wild type Aequoria GFP, states "the difference in *fluorescene was not sufficient* to resolve infected from uninfected cells" *[sic]* (Emphasis provided by appellants; Brief, p. 8, footnote 13).

Appellant's citation 1 is only referring to one embodiment of the reference's teaching where only "a single copy of the gene is stably integrated". The statement does not indicate that a retroviral vector comprising the gene encoding for the wildtype GFP, in general, would not express at all. (Disclosed examples and preferred embodiments do not constitute a teaching away from a broader disclosure or nonpreferred embodiments. *In re Susi*, 440 F.2d 442, 169 USPQ 423 (CCPA 1971). "A known or obvious composition does not become patentable simply because it has been described as somewhat inferior to some other product for the same use." *In re Gurley*, 27 F.3d 551, 554, 31 USPQ2d 1130, 1132 (Fed. Cir. 1994)).

In regard to Appellant's citation 2, the complete teaching of the reference is as follows:

"While the median fluorescence value of the MGF-wtGFP-infected population was 2-fold greater than that of uninfected cells (Fig. 1A), the difference in fluorescence was not sufficient to resolve infected from uninfected cells." (p. 8509, left col., para 4, bottom; emphasis added).

(It is noted that MGF-wtGFP represents the retroviral vector used to infect the mammalian cell for GFP expression).

Although, the difference in fluorescence value between the infected and the uninfected cells are not statistically significant, the wildtype GFP still expressed as indicated by the 2-fold increase in fluorescence value. Overall, not only does the Anderson reference demonstrate the success of generating retroviral vector comprising polynucleotides that encode for wild-type GFP, but the reference also demonstrates the successful generation of mammalian cells comprising the said retroviral vector.

**Rejection over Bryan and Aran:**

Appellants mainly argue that the Aran reference discloses at page 204 of the reference "a retroviral vector encoding a wild-type *Aequoria* GFP was introduced into in a mammalian cell, fluorescence was **"undetectable"**. As such, Aran's disclosure, itself, teaches that the combination of Bryan and Aran would produce "a seemingly inoperative" vector.[13]" [*sic*] (Brief, p. 11, para 3; emphasis provided by appellants).

Appellants are basing the lack of "reasonable expectation of success" on the intended use of the claimed product. The "wild-type Aequoria GFP" referred to by the Aran reference is wild-type both in terms of the amino acid sequence and the encoding polynucleotide sequence. The reference also teaches that the humanized (in terms of the polynucleotide codons) GFP have exhibit higher fluorescent activity (Aran, p. 204, left col., para 2). More importantly, the reference teaches the successful generation of a retroviral vector comprising a polynucleotide encoding for the wild-type GFP (Aran, p. 196-197). Thus, the reference does not teach that a retroviral vector comprising a polynucleotide encoding for the wild-type Renilla GFP cannot be made or used.

**Rejection over Aran, Bryan and Zolutukhin:**

Applicants traversed this rejection with the same argument over the combination of Bryan and Aran rejection.

**Rejection over Zolutukhin and Bryan:**

Applicants traversed this rejection by referring to the "general discussion" (or the "Arguments directed to all rejections" section of the instant Appeal Brief. Appellants' arguments in the "general discussion" section are as answered above.

**Rejection over Bierhuizen and Bryan:**

Applicants traversed this rejection by referring to the "general discussion" (or the "Arguments directed to all rejections" section of the instant Appeal Brief. Appellants' arguments in the "general discussion" section are as answered above.

Appellants further traversed this rejection by the following arguments:

1. The Bierhuizen is "only a single reference in a field in which many others report repeated failure." (Brief, p. 12, para 8)

2. "Bierhuizen, in fact, reports only marginal results in their vector." (Brief, p. 12, para 8); Appellants specifically argue that "Bierhuizen fails to report stable cell lines that express wild type *Aequoria* GFP." *[sic]*(Brief, p. 13, para 1).

In regard to appellants' argument 1., the "supporting references" cited by appellants do not indicate failure of generating a retroviral vector encoding a wild-type Aequoria GFP, as discussed above (under the section "Discussion of Appellant's "Supporting References") Furthermore, the success of Beirhuizen would invite or motive one of ordinary skill in the art to experiment with wild-type GFP by generating retroviral vectors comprising polynucleotides encoding for wild-type GFP.

Appellants' argument 2 is based on a feature (i.e. "stable cell lines") that is not recited in the instant specification. In response to applicant's argument that the references fail to show certain features of applicant's invention, it is noted that the features upon which applicant relies (i.e., stable cell line expressing wild-type Aequoria GFP) are not recited in the rejected claim(s). Although the claims are interpreted in light of the specification, limitations from the specification are not read into the claims. See *In re Van Geuns*, 988 F.2d 1181, 26 USPQ2d 1057 (Fed. Cir. 1993).

**Rejection over Bierhuizen, Bryan and Aran:**

Appellants traversed this rejection with the same arguments as the above rejections regarding the Aran and Bierhuizen references.

**(11) Related Proceeding(s) Appendix**

No decision rendered by a court or the Board is identified by the examiner in the Related Appeals and Interferences section of this examiner's answer.

For the above reasons, it is believed that the rejections should be sustained.

Respectfully submitted,

Sue Liu
Examiner
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SL

6/27/07

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